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SAFETY TESTING OF DENGUE-1 AND DENGUE-3 SEEDS FOR HUMAN CHALLENGES, UNATTENUATED; HEPATITIS A VIRUS, STRAIN HM-175

FINAL REPORT

By

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June 1989

Supported by

U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND

Fort Detrick, Frederick, Maryland 21701-5012

CONTRACT NO. DAMD17-86-C-6188

Flow Laboratories, Inc.
McLean, Virginia 22102

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SECURITY CLASSIFICATION OF THIS PAGE

REPORT DOCUMENTATION PAGE

Form Approved OMB No. 0704-0188

1a. REPORT SECURITY CLASSIFICATION UNCLASSIFIED	1b. RESTRICTIVE MARKINGS
2a. SECURITY CLASSIFICATION AUTHORITY	3. DISTRIBUTION/AVAILABILITY OF REPORT Approved for public release; distribution unlimited
2b. DECLASSIFICATION/DOWNGRADING SCHEDULE	
4. PERFORMING ORGANIZATION REPORT NUMBER(S)	5. MONITORING ORGANIZATION REPORT NUMBER(S)
6a. NAME OF PERFORMING ORGANIZATION Flow Laboratories, Inc. 6b. OFFICE SYMBOL (If applicable)	7a. NAME OF MONITORING ORGANIZATION
6c. ADDRESS (City, State, and ZIP Code) McLean, Virginia 22102	7b. ADDRESS (City, State, and ZIP Code)
8a. NAME OF FUNDING/SPONSORING ORGANIZATION U.S. Army Medical Research & Development Command 8b. OFFICE SYMBOL (If applicable) SGRD-RMI-S	9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER DAMD17-86-C-6188
8c. ADDRESS (City, State, and ZIP Code) Fort Detrick Frederick, Maryland 21701-5012	10. SOURCE OF FUNDING NUMBERS PROGRAM ELEMENT NO. 62770A; PROJECT NO. 3M1-62770A870; TASK NO. AC; WORK UNIT ACCESSION NO. 029

11. TITLE (Include Security Classification)
(U) Safety Testing of Dengue-1 and Dengue-3 Seeds for Human Challenges, Unattenuated; Hepatitis A Virus, Strain HM-175

12. PERSONAL AUTHOR(S)
Potash, Louis

13a. TYPE OF REPORT	13b. TIME COVERED	14. DATE OF REPORT (Year, Month, Day)	15. PAGE COUNT
Final	FROM 86May 1 TO 89Apr30	1989 June	9

16. SUPPLEMENTARY NOTATION

17 COSATI CODES		
FIELD	GROUP	SUB-GROUP
06	13	
06	03	

18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)
Dengue-1; Dengue-3; Hepatitis A; Viral seeds; Vaccine; PA-1

19. ABSTRACT (Continue on reverse if necessary and identify by block number)

Tests of seed and vaccine materials for safety in humans consisted of inoculation in: 1) five different cell culture lines; 2) mice (adult and new-born sucklings), guinea pigs and rabbits; and 3) bacterial, fungal and mycoplasma culture media. The inocula for these tests included crude, unclarified harvests of both control and virus fluids (neutralized and/or un-neutralized). Tested were the following fluids: unattenuated dengue-1, dengue-3 and dengue-4 virus seed lots, a Japanese encephalitis (JE) production seed and vaccine, a hepatitis A vaccine. Tests of the Final Products included: 1) sterility; 2) inocuity in mice and guinea pigs; and 3) reverse transcriptase activity. Final Products of a JE and a nepatitis A vaccine were tested. All tests were carried out following the guidelines established by the FDA for live and inactivated virus vaccines as found in 21 CFR, Part 600 and were performed in accordance with GLP regulations. In an effort to attenuate a dengue-1 vaccine lot, the virus was subjected to 10 serial passages in dog kidney cell cultures,

20. DISTRIBUTION/AVAILABILITY OF ABSTRACT ☐ UNCLASSIFIED/UNLIMITED ☒ SAME AS RPT ☐ DTIC USERS	21. ABSTRACT SECURITY CLASSIFICATION UNCLASSIFIED
22a. NAME OF RESPONSIBLE INDIVIDUAL Mary Frances Bostian	22b. TELEPHONE (Include Area Code) 301/663-7325 22c. OFFICE SYMBOL SGRD-RMI-S

DD Form 1473, JUN 86 Previous editions are obsolete. SECURITY CLASSIFICATION OF THIS PAGE

SUMMARY

The Virus Vaccine Production Laboratory of Flow Laboratories, Inc. has been under a USAMRDC contract to provide research services entitled "Safety Testing of Dengue-1 and Dengue-3 Seeds for Human Challenges, Unattenuated". The services, involving tests of seed and vaccine materials for safety in humans, consisted of inoculation in: 1) five different cell culture lines; 2) mice (adult and new-born sucklings), guinea pigs and rabbits; and 3) bacterial, fungal, and mycoplasma culture media. The inocula for these tests included crude, unclarified harvests of both control and virus fluids (neutralized and/or un-neutralized). The final product(s) was tested for sterility, inocuity in mice and guinea pigs, and for reverse transcriptase activity. All tests were carried out following the guidelines established by the FDA for live and inactivated virus vaccines as found in 21 CFR, Part 600 and were performed in accordance with GLP regulations.

During this 3 year contract, safety tests were performed on the following fluids: unattenuated dengue-1, dengue-3 and dengue-4 virus seed lots, a Japanese encephalitis (JE) production seed, vaccine and Final Product, and on a hepatitis A vaccine and Final Product. Phase Reports, after review and corrections, were distributed in accordance with contract specifications. In addition, a dengue-1 vaccine lot was serially passaged 10 times in dog kidney (DK) cell cultures in an effort to attenuate the virus. Aliquots of each passage level were submitted to the COR, as directed.

FOREWORD

In conducting the research described in this report, the investigator(s) adhered to the Guide for the Care and Use of Laboratory Animals prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (DHHS, PHS, NIH Publications No. 85-23, Revised 1985).

I. INTRODUCTION

This Final Report for the research period of May 1, 1986 to April 30, 1989 summarizes the laboratory activities performed at Flow Laboratories, Inc. under contract - DAMD17-86-C-6188.

All Phase Reports were written in a form satisfactory for submission to the Office of Biologics (OOB), Federal Drug Administration (FDA) for review as an Investigational New Drug (IND). In accordance with the Good Laboratory Practices (GLP) regulations as stated in the Code of Federal Regulations (CFR) Number 21, Part 58.35(b)(6), a post release inspection of the records of each of the interim Phase Reports was made by the Quality Assurance (QA) Auditor. The QA reports indicated that, in conducting the tests described in these Reports, the investigator(s) operated within compliance with applicable sections of the GLP regulations (21 CFR, Part 58) and followed the guidelines established by the FDA for live and inactivated virus vaccines as found in 21 CFR, Parts 610.11, 610.12, 610.30, 630.10 - 630.17, etc. All test procedures are detailed in formal Standard Operational Procedures (SOPs) with observations recorded and initialed on designated laboratory Forms.

II. SAFETY TESTING

Over the 3 year period, a total of 5 different virus fluids plus related control fluids were safety tested. As required by the FDA guidelines, all unclarified harvest fluids were tested for:

a) microbial sterility [bacterial, fungal, mycobacterial and mycoplasmal];
b) purity (safety) in tissue cultures [4 tissue culture systems plus the cell system in which the virus was grown];
c) animal safety in adult mice, newborn suckling mice, rabbits and guinea pigs; and, when required,
d) embryonated eggs via allantoic and yolk sac inoculations.

All Final Products were tested for:

a) microbial sterility [bacterial and fungal];
b) general safety in mice and guinea pigs; and
c) reverse transcriptase activity.

The 5 viruses tested consisted of three (3) dengue virus challenge seeds (non-attenuated) of both unclarified harvests and freeze-dried Final Products, a Japanese encephalitis virus (JE) unclarified production seed and vaccine harvests and freeze-dried Final Product Vaccine, and a Hepatitis A virus, strain HM-175, unclarified vaccine harvests and formalin-inactivated, alum-adsorbed Final Product Vaccine.

DENGUE VIRUSES

Dengue Virus Type 1 (non-attenuated), Strain: Western Pacific 1974, LOT No.1 of February 1984. The initial tissue culture purity (safety) test in AGMK cell cultures was unsuccessful and was attributed to the failure of the supplied antiserum to completely neutralize the undiluted virus in this cell system. However, pre-treatment of the secondary cell cultures with the specific antiserum prior to inoculation prevented/inhibited the dengue virus-breakthrough (non-descript CPE) but permitted the expression of the typical CPE following challenge with Coxsackie A-9 virus (an assay for the detection of non-CPE producing and or latent agents via the interference phenomenon). This fluid was considered to have passed satisfactorily all the prescribed tests for safety, including purity. The Phase Report dated November 1, 1986, after approval and corrections, was distributed on March 4, 1987.

Dengue Virus Type 3 (non-attenuated), Strain: CH-53489, LOT No.1 of April 1984. The tissue culture purity (safety) test in AGMK cell cultures was unsuccessful and was attributed to the failure of the supplied antiserum to completely neutralize the undiluted virus in this cell system. Even after multiple attempts which included repeated treatments with the specific antiserum and which finally resulted in the prevention/inhibition of the dengue virus-breakthrough (non-descript CPE), the Coxsackie A-9 virus challenge CPE was still completely inhibited. Other than this one assay, the fluid was considered to have passed satisfactorily all the prescribed tests for safety, including purity. The Phase Report dated March 16, 1987, after approval and corrections, was distributed on June 12, 1987.

Dengue Virus Type 4 (non-attenuated), Strain: Carib 341750, LOT No. 2 of Jan 1986. The tissue culture purity (safety) tests in all 5 tissue culture systems were considered unsatisfactory when all cultures inoculated with the serum-virus mixture were found to readily hemagglutinate guinea pig RBCs. It was subsequently determined that the hyperimmune rabbit serum alone readily hemagglutinated guinea pig RBCs suggesting that the serum may have been contaminated with a hemagglutinating agent(s). The Phase Report dated July 1, 1988, after approval and corrections, was distributed on September 30, 1988.

JAPANESE ENCEPHALITIS (JE) VIRUS

Japanese Encephalitis (JE), Strain SA14-14-2, Production Seed Virus LOT No. PDK8-WR2, Mfg. Date Dec 86 and Live Attenuated Virus Vaccine LOT No. PDK-9-WR3, Mfg. Date Feb 87. Utilizing the testing procedures listed above including the assay in embryonated eggs, these fluids were found to have passed satisfactorily all the prescribed tests for safety, including purity. The Phase Report dated October 26, 1987, after approval and corrections, was distributed on December 10, 1987.

HEPATITIS A VIRUS

Hepatitis A Virus, Strain HM-175, vaccine FI-2, Lot1A, day 31 harvest, unclarified of 16 Oct 87 and Lot 1B, day 31 harvest, unclarified of 4 Dec 87. As the individual volumes of Lots IA and IB available were insufficient to perform all the prescribed tests, a single pool was prepared of the two lots. Utilizing the testing procedures listed above (minus the assay in embryonated eggs), this pool was found to pass satisfactorily all the prescribed tests for safety, including purity. The Phase Report dated July 1, 1988, after approval and corrections, was distributed on November 28, 1988.

Hepatitis A Virus Vaccine, Strain HM-175, FI-2, Lot No. 1 of Jan 89 - - (5 ml Inactivated with 0.05% Formalin, Adsorbed with Alum, containing preservative: 0.375% phenoxyethanol. This Final Container Vaccine satisfactorily passed the tests for Microbial Sterility and General Safety. The Phase Report dated March 21, 1989, after approval and corrections, was distributed on April 26, 1989.

III. SERIAL PASSAGES

Dengue Virus Type 1, Strain 45AZ5: Live-Attenuated Vaccine, Lot No. 1-82, Run 2. As an adjunct to these Safety Tests, this laboratory undertook the study to subject this vaccine to 10 serial passages in dog kidney cell cultures in an effort to attenuate this virus. Pre-screened, frozen ampules of primary dog kidney [PDK] cells (Lot 222) were supplied by the COR. All studies were carried out in accordance with the protocols submitted by the COR and included passaged control cultures. The laboratory successfully completed the 10 serial passages using both 1st and 2nd passage DK cell cultures with multiple 2 ml vials of each passage level (day 7 harvests of both virus infected and control cultures) submitted to the COR. In addition, bulk volumes of the same harvests from the 2nd through 10th passage levels are being stored at Flow's facility to serve as possible back-up, if needed.

CONCLUSIONS

A major problem that became very evident while attempting to perform the tissue culture purity (safety) tests with the dengue viruses, in particular, was the lack of high-titered, specific immune serum. For any future safety tests of the dengue viruses (whether attenuated vaccines or challenge seeds), it is imperative that specific, high-titered immune serum be available for use. It should be of interest that Flow Laboratories, using special proprietary technology of a partner company, is capable of producing high titered, specific antibody in as few as a single rabbit while employing minimal amounts of purified antigen. With the recent awarding of a new 3 year contract to Flow - DAMD17-89-C-9119, the production of the dengue antisera should proceed as soon as the required purified antigens are made available.

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